ORIGINAL ARTICLE

Prevalence and determinants of *Chlamydia trachomatis* infections in women from Bogota, Colombia

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Objectives: *Chlamydia trachomatis* infection in the cervix and uterus has been hypothesised to be a co-factor for cervical cancer. We performed a cross sectional study in Bogota, Colombia, where cervical cancer rates are high, to determine the prevalence and determinants of *C trachomatis* infection, and in particular its association with human papillomavirus (HPV).

Methods: 1829 low income sexually active women were interviewed and tested for *C trachomatis*, using an endogenous plasmid PCR-EIA, and for 37 HPV types, using a general primer GP5+/6+ mediated PCR-EIA.

Results: The overall prevalence of *C trachomatis* was 5.0%, and it did not differ substantially between women with normal (5.0%) and those with abnormal (5.2%) cervical cytology. Women infected with any HPV type (15.1%) had a slightly increased risk of being simultaneously infected with *C trachomatis* (adjusted OR 1.3, 95% CI: 0.8 to 2.4). This association was stronger when multiple HPV infections (adjusted OR 2.5, 95% CI: 1.1 to 5.9) were present. No other lifestyle or reproductive characteristics were clearly associated with risk of *C trachomatis* infection.

Conclusions: HPV infected women, particularly women with multiple HPV infections, are at increased risk of being infected with *C trachomatis*.

Infections with *Chlamydia trachomatis*, a highly prevalent sexually transmitted agent worldwide, are mostly asymptomatic (70%–80%) and often remain undetected. Besides causing cervicitis and urethritis, these infections may result in serious secondary complications, such as pelvic inflammatory disease and pelvic pain (18%–24%), tubal infertility (6%–21%), and ectopic pregnancy (in 7%–9% of those who become pregnant).[1–5] In addition, *C trachomatis* has been suggested to be a cofactor in the development of cervical cancer.[6]

In Colombia, the prevalence and determinants of *C trachomatis* infection, in particular its association with HPV infections, have not yet been described.

We report here on the prevalence and determinants on *C trachomatis* infection in a large sample from a low income population in Bogota, Colombia.

PATIENTS AND METHODS

Study population

A prospective cohort study in Bogota, Colombia, was initiated in the early 1990s by the Colombian National Institute of Cancer and the International Agency for Research on Cancer (IARC). The aim of the study was to investigate the natural history of human papillomavirus (HPV) infections and cervical intraepithelial neoplasias (CIN) lesions among low income women.

From November 1993 to November 1995, the Colombian National Cervical Cancer Institute conducted a census in four health districts in Bogota, which had no cervical screening programme previously implemented. Women aged 18–44 years were initially invited to participate. Subsequently, the upper age limit was expanded to screen elderly women also.

For the study presented here, the first 2000 women registered in the above census were invited to participate. They were interviewed face to face by specially trained interviewers and answered a structured questionnaire on sociodemographic characteristics, lifelong sexual behaviour, reproductive history, smoking, and dietary habits. After interview they were offered a cervical examination, when a Pap smear was collected, and asked to donate a blood sample. Only women who had ever been sexually active were included for this study.

Informed consent was obtained from all participants included in the study. The local ethics committee and the ethics committee at IARC cleared the study protocol.

Biological specimens

Cervical scrapes were collected from each woman using two Ayre spatulas and two endocervical brushes. The first spatula and brush were used for routine Pap smear, which were classified according to the Bethesda system. The second spatula and brush, and the remaining cells of the first spatula and brush, were placed in a tube containing 5 ml of phosphate buffered saline (PBS 1X) +0.05% thiomersal. Cells were detached from the spatula and endocervical brush by vortexing, and centrifuged at 3000 *g* for 10 minutes. The cell pellet was resuspended in 1 ml buffer 10 mM TRIS-HCI pH 8.3 and stored at −70°C until use. For analysis, a 100 µl aliquot was boiled for 10 minutes at 100°C, cooled on ice, and centrifuged for 1 minute at 3000 *g*. 10 µl of this pretreated crude cell suspension was used for polymerase chain reaction (PCR) analysis.[7 8]

In order to assess the quality of the target DNA, a 209 base pair amplifying β globin PCR was performed using the primer combination BGPCO3 and BGPCO5 as previously described.[9]

C trachomatis detection by PCR

The detection of *C trachomatis* was performed as described previously.[10] Plasmid endogenous specific primers Bio

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PL6.1 (Bio 5′-AGAGTACATCGGTCAACGA-3′) and PL6.2 (3-TCACAGCGGTTGCTCGAAGCA-5′) were used for PCR amplification. Briefly, the reaction was carried out in 50 µl of PCR solution containing 10 mM TRIS-HCl pH 8.3, 50 mM KCl, 200 µM of each deoxynucleotide, 1.5 mM of $MgCl_2$, 1 U of DNA polymerase (AmpliTaq; Perkin-Elmer, USA), 25 pmol of each primer (Eurogentec, Belgium), and 5 µl of sample. The PCR amplification consisted of DNA denaturation at 95℃ for 4 minutes followed by 40 cycles of amplification using a PE 9600 thermocycler (Perkin-Elmer, USA). Each cycle included a denaturation step at 95℃ for 1 minute, one annealing step at 55℃ for 1 minute, and a chain elongation step at 72℃ for 1.5 minutes. The final elongation step was extended for another 4 minutes.

The biotinylated PL6.1/PL6.2 PCR products were detected using an enzyme immunoassay as described previously.[11 12] Briefly, in this assay, 5 µl of the biotinylated PCR products were captured for 1 hour incubation in streptavidin coated wells of a microtitre plate (Roche, Mannheim, Germany). Subsequently, the wells were washed three times with 1X SSC; the captured DNA was denaturated by alkaline treatment with 0.1 M NaOH, and hybridised for 1 hour using the digoxigenin labelled type specific probe to PL6.1/PL6.2 amplified products. The unbound probe was removed by washing three times with 1X SSC and the hybrids were detected using an anti-dig Fab framents labelled with alkaline phosphatase (Roche, Mannheim, Germany) and paranitrophenyl phosphate (Sigma, USA) was used as substrate. Finally, the optical density (OD) was measured at 405 nm using a Labsystem Multiscan reader. In our assay a cut-off point was defined using three times mean OD of the negative controls. As a positive control, a 10-fold dilution series of *C trachomatis* L2 DNA was used as previously described,[13] resulting in a detection sensitivity corresponding to 0.01–0.10 inclusion forming units (IFU).

HPV detection by PCR

HPV-DNA detection in these samples had been performed previously by a standard GP5+/GP6+ PCR-EIA based assay,[9] and HPV results for women with cytomorphologically normal scrapes are presented elsewhere.[14] In the present report, HPV results of abnormal cytology are additionally presented.

Briefly, HPV positives samples were subjected to EIA-HPV group specific analysis using cocktail probes for high risk (HR) and low risk (LR) HPVs.[11] The HR HPV cocktail probe consisted of oligoprobes for HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The LR HPV consisted of oligoprobes for HPV 6, 11, 40, 42, 43, 44, HPV82 (MM4), HPV 83 (MM7), HPV 84 (MM8), Iso39, HPV 71 (CP8061), CP6108, HPV 81 (CP8304), HPV 26, 34, 53, 54, 55, 57, 61, 70, 72, and 73. However, HPV types with unknown oncogenic potential—namely, 26, 34, 53, 73, and Iso39—were considered in the results as HR types. This was based on both the alignment analysis of the E6 gene (modified from Myers *et al*[15]) and the risk estimates obtained for the various HPV types within a multicentre case-control study of cervical cancer conducted by the IARC.[16–23] Additionally, HPV positivity was assessed by Southern blot hybridisation of GP5+/GP6+ PCR products with the general probe of specific (α^{-32} P)dCTP labelled DNA fragments from cloned DNA of HPV 6, 11, 16, 18, 31 and 33.[7 9] Samples that were positive by Southern blot analyses and negative by HR/LR EIA were considered as HPV X or undetermined type.

Statistical analysis

The association between *C trachomatis* infections and different risk factors was evaluated in all women regardless of their cytological diagnosis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression models, considering *C trachomatis* infections as a dependent variable, and several known or hypothesised risk factors for cervical cancer as independent variables. The software used for data management and analysis was STATA (Stata Press, College Station, TX, USA). We performed both age adjusted (with age grouped as <25, 25–29, 30–34, 35–44, 45 or more years) and multivariate analyses. The following variables were included in the multivariate models: age (as categorised above), educational level (low, education up to primary level; intermediate, at least half of secondary school or complete secondary school; and high, technical school or university), number of lifetime regular (1, 2, 3 or more) and casual (yes or no) sexual partners, parity (nulliparous, 1–2 children, 3 or more), age first sexual intercourse (<17, 17–19, 20 or more years), oral contraceptive use (ever or never), condom use, and smoking patterns (ever smoked at least 100 cigarettes). No sample size calculation was done a priori for this study.

RESULTS

From the 2000 invited women, 53 refused to participate, seven were considered ineligible (mental illness, hysterectomy, history of cervical cancer), 29 did not provide cell specimens for HPV detection, and 82 had poor DNA quality as indicated by negative β globin PCR and were therefore considered inadequate for HPV-PCR and *C trachomatis* PCR testing. Thus, a total of 1829 women had PCR and valid HPV and *Chlamydia* results. However, for the analysis of characteristics of the studied population we had to further exclude 16 women who did not answer the questionnaire completely.

Cytology

All Pap smears were initially classified both using the CIN classification and the Bethesda System terminology. In all, 1687 women (92.2%) had normal cytological findings (table 1). Of the 115 women with abnormal cytology, 63 (3.4%) were diagnosed with low grade squamous intraepithelial lesion (LSIL), 28 (1.5%) had atypical squamous cell of undetermined significance (ASCUS), 14 (0.8%) had atypical glandular cells of undetermined significance (AGUS), eight (0.4%) had high grade squamous intraepithelial lesions (HSIL), and two (0.1%) had invasive cervical cancer.

Table 1 HPV and *C trachomatis* infections and cytological diagnosis in women from Bogota, Colombia

	Normal		Abnormal		Inadequate		Any cytology	
Cytology	No	%	No	%	No	%	No	%
Total study population	1687	92.2	115	6.3	27	1.5	1829	100.0
HPV positive	229	13.6	45	39.1	3	11.1	277	15.1
C trachomatis positive	85	5.0	6	5.2	1	3.7	92	5.0

% in the population line shows the distribution of cytological diagnosis in the studied population.
% in the HPV and *C trachomatis* lines show the prevalence of infection in each diagnosis group.

Characteristics of the studied women

The median age of women participating in the study was 33 years (range 18–85 years). Most women had low (38.0%) or intermediate (36.9%) educational level, had their first sexual intercourse or first regular sexual partner before the age of 20 (62.2%), reported only one lifelong sexual partner (74.0%), and had at least two full term pregnancies (median 2). IUD was the most common contraceptive method ever used (59.1%), followed by oral contraceptives (49.9%) and condoms (31.6%). Less than one third (28.3%) had ever smoked regularly (table 2).

C trachomatis prevalence and risk factors

The overall prevalence of *C trachomatis* infection was 5.0%, and did not differ substantially between women with normal or abnormal cytological results (table 1).

The highest prevalence of *C trachomatis* was observed in women aged 30–34 years (7.9%) and the lowest in women older than 45 years (1.5%) (table 2; fig 1).

Table 2 Determinants of *C trachomatis* infection in women from Bogota, Colombia

	Total number of women (n = 1813)*		Women infected with *C trachomatis*			
	No	%	No	%	OR†	OR‡
Age (y)						
<25	293	16.2	19	6.5	1.7 (0.87 to 3.1)	1.6 (0.7 to 3.5)
25–29	360	19.9	14	3.9	1.0 (0.5 to 1.9)	0.8 (0.4 to 1.8)
30–34	433	23.9	34	7.9	2.0 (1.2 to 3.6)	1.8 (0.98 to 3.2)
35–44	522	28.8	21	4.0	1	1
45+	205	11.3	3	1.5	0.4 (0.1 to 1.2)	0.2 (0.06 to 1.1)
Education						
None/low	689	38.0	31	4.5	1	1
Intermediate	668	36.9	30	4.5	0.8 (0.4 to 1.3)	0.8 (0.4 to 1.4)
High	455	25.1	30	6.6	1.3 (0.7 to 2.0)	1.2 (0.7 to 2.5)
Missing	1	0.06	0	0.0		
Age at 1st sexual intercourse (y)						
⩾20	686	37.8	27	3.9	1	1
17–19	633	34.9	39	6.2	1.5 (0.92 to 2.6)	1.3 (0.8 to 2.3)
⩽16	494	27.3	25	5.1	1.3 (0.7 to 2.2)	1.3 (0.6 to 2.4)
Lifetime number of regular sexual partners§						
1	1341	74.0	66	4.9	1	1
2	309	17.0	14	4.5	0.9 (0.5 to 1.7)	0.9 (0.5 to 1.6)
3	62	3.4	3	4.8	1.1 (0.3 to 3.7)	1.0 (0.3 to 3.3)
Missing	101	5.6	8	7.9		
Ever had a casual sexual partner¶						
No	1302	71.8	61	4.7	1	1
Yes	428	23.6	23	5.4	1.1 (0.7 to 1.8)	1.0 (0.6 to 1.8)
Missing	83	4.6	7	8.4		
Parity						
0	115	6.3	6	5.2	1	1
1–2	944	52.1	52	5.5	1.1 (0.4 to 2.6)	1.5 (0.5 to 4.4)
⩾3	754	41.6	33	4.4	1.0 (0.4 to 2.6)	1.5 (0.5 to 5.0)
Oral contraceptives						
Never used	886	48.9	48	5.4	1	1
Former user	741	40.9	30	4.1	0.7 (0.4 to 1.2)	0.7 (0.4 to 1.2)
Current user	163	9.0	12	7.4	1.2 (0.6 to 2.3)	1.4 (0.7 to 2.7)
Missing	23	1.3	1	4.4		
Duration of oral contraceptive use						
<3 years	544	30.0	21	3.9	0.7 (0.4 to 1.1)	0.7 (0.4 to 1.2)
⩾3 years	287	15.8	17	5.9	1.1 (0.6 to 2.0)	1.2 (0.6 to 2.1)
Missing duration	73	4.0	4	5.5		
IUD						
Never used	710	39.2	36	5.1	1	1
Former user	572	31.6	29	5.1	1.0 (0.6 to 1.7)	1.1 (0.6 to 1.8)
Current user	500	27.6	26	5.2	0.9 (0.5 to 1.5)	0.9 (0.5 to 1.5)
Missing	31	1.7	0	0.0		
Condom						
Never	1212	66.9	59	4.9	1	1
Ever	572	31.6	32	5.6	1.1 (0.7 to 1.7)	1.0 (0.7 to 1.9)
Missing	29	1.6	0	0.0		
Smoking						
Never	1300	71.7	61	4.7	1	1
Former smoker	215	11.9	10	4.7	1.1 (0.5 to 2.2)	1.1 (0.6 to 2.3)
Current smoker	298	16.4	20	6.7	1.4 (0.8 to 2.4)	1.1 (0.6 to 2.1)

*1843 women with complete questionnaire information and *C trachomatis* detection; 16 women who did not fill in completely the questionnaire were excluded.
†OR = odds ratio adjusted for age.
‡OR = odds ratio adjusted for age, education, number of regular partners, casual partners, parity, age at first sexual intercourse, use of oral contraceptives and condom, and smoking habit.
§Defined as sexual partners in relationships lasting at least 6 months.
¶Defined as sexual partners in relationships lasting less than 6 months.

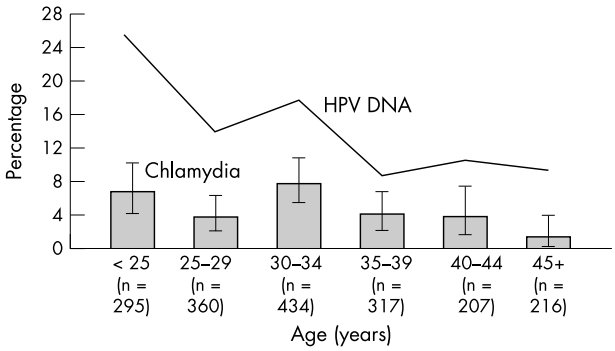

Figure 1 Prevalence and 95% CI of *C trachomatis* and HPV DNA in women from Bogota, Colombia.

There was no clear association between educational level, sexual behaviour (number of regular or casual sexual partners), reproductive history (parity, ever use or duration of use of oral contraceptives, use of condoms or IUD), or smoking patterns and risk of *C trachomatis* infection (table 2).

HPV prevalence

The overall HPV DNA prevalence was 15.1%. Women presenting abnormal cytology had a substantially higher prevalence of HPV infections (39.1%) than women with normal cytology (13.6%) (table 1).

There were more women infected with HR HPV types (11.9%) than with LR types (2.9%). Infections with uncharacterised types (HPV X, 0.5%) were few (table 3).

Association between *C trachomatis* and HPV infections

The prevalence of *C trachomatis* infections was non-significantly higher among women infected with HPV of any studied type than among HPV uninfected women (6.9% *v* 4.7%; adjusted OR 1.3, 95% CI: 0.8 to 2.4). *C trachomatis* prevalence was higher among women with multiple HPV infections (9.5%) than women with single HPV infections (5.9%; adjusted OR 2.5, 95% CI: 1.1 to 5.9) (table 3).

DISCUSSION

This is the first study describing the prevalence and determinants of *C trachomatis* infections in the population of Bogota, Colombia.

The overall *C trachomatis* prevalence in this study (5.0%) was similar to the prevalence reported in women from the general population in Amsterdam, Colorado, Washington, and Copenhagen, which ranged from 4.5% to 9%.[12 24–26] Our results also confirm a somewhat higher *C trachomatis* infection prevalence in young age groups, as observed in other populations.[12 27] Behavioural factors (such as early age in the initiation of sexual activity, multiple partners, irregular use of condoms, use of contraceptive methods) were, unexpectedly, not associated with *C trachomatis* infection risk. Thus, our study is in partial disagreement with some previous reports,[12 25 28 29] which found associations with *C trachomatis* infection and oral contraceptive use, parity, lifetime number of sexual partners, and smoking habits. Discrepancies between our results and those from studies conducted elsewhere may be because of differences in the characteristics of the studied populations (such as age, patterns of sexual behaviour or openess in reporting sexual behaviour), and variations in the sensitivity and specificity of laboratory methods used.

Duration of oral contraceptive use had no apparent influence on the prevalence of *C trachomatis* infection—as reported also by Munk *et al*[12] and Jacobson *et al*.[30] The lack of association between parity and *C trachomatis* infection observed by us is in line with the study by Munk *et al*[12] but it contrasts with the study by Bagshaw *et al*.[31]

Besides young age, the only risk factor independently associated with *C trachomatis* infection in our study was HPV infection, particularly multiple infections. This is in agreement with some previous studies using PCR and serological assays.[32 33] The clear association between *C trachomatis* and multiple HPV infections might be indicative of multiplicity of sexual partners from women themselves or their partners. The male partner(s) sexual practices (such as multiplicity of sexual partners or high risk sexual behaviour) is a plausible explanation for *C trachomatis* infection among women. Such information is, however, not available in our study.

Some studies reported an association between antibodies to *C trachomatis* and SIL lesions.[34–37] However, analysis of the DNA presence of *C trachomatis* and its association with HPV infections and SIL lesions is still limited. We did not observe any substantial difference in *C trachomatis* infection prevalence between women with normal or abnormal cervical cytology. This suggests that *C trachomatis* does not itself induce cervical abnormalities. The role of *C trachomatis* as a potential cofactor in cervical disease, however, cannot be ruled out.

Table 3 *C trachomatis* infection and HPV infection in women from Bogota, Colombia

	Total number of women (n = 1813)*		Women infected with *C trachomatis*			
	No	%	No	%	OR†	OR‡
HPV negative (uninfected)	1536	84.7	72	4.7	1	1
HPV positive (any type)	277	15.3	19	6.9	1.3 (0.8 to 2.3)	1.3 (0.8 to 2.4)
HPV types§						
High risk	16	11.9	14	6.5	1.2 (0.7 to 2.3)	1.3 (0.7 to 2.4)
Low risk	52	2.9	3	5.8	1.1 (0.3 to 3.8)	0.9 (0.2 to 3.8)
HPV X	9	0.5	2	22.2	4.6 (0.9 to 23.0)	4.6 (0.9 to 24.2)
Single infection	203	11.2	12	5.9	1.1 (0.6 to 2.1)	1.0 (0.5 to 2.0)
Multiple infections	74	4.1	7	9.5	2.0 (0.9 to 4.6)	2.5 (1.1 to 5.9)

*1843 women with complete questionnaire information and *C trachomatis* detection; 16 women who did not fill in completely the questionnaire were excluded.
†OR = odds ratio adjusted for age.
‡OR = odds ratio adjusted for age, education, number of regular partners, casual partners, parity, age at first sexual intercourse, use of oral contraceptives and condom, and smoking habit.
§High risk (HR) types: 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, ISO39, 73. Low risk (LR) types: 6, 11, 40, 42, 43, 44, MM4, MM7, MM8, CP8061, CP6108, CP 8304, 54, 55, 57, 61, 70, 72.

Key messages

- *C trachomatis* infection is relatively common (about 5% prevalence) among adult women in Bogota, Colombia.
- HPV infected women, particularly women with multiple HPV infections, are at increased risk of being infected with *C trachomatis*.
- The overall prevalence of *C trachomatis* does not seem to differ substantially between women with normal and those with abnormal cervical cytology.

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CONTRIBUTORS

MM was responsible for laboratory testing and participated in the interpretation of results; EW, SF, and AA were responsible for statistical analysis, interpretation of the results and drafting of the manuscript; EW wrote the final version of the paper; HP and MR coordinated the study design and inclusion of patients; NM coordinated the overall study design and participated in drafting of the paper; SM participated in laboratory testing; CJLMM and AJCB coordinated the laboratory testing. The HPV Study Group participated in the inclusion of patients and cytology laboratory analysis.

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